

Standard Article

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Survey of Two New (Kai 1 and Kai 2) and Other Blood Groups in Dogs of North America

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Background: Based upon serology, >10 canine blood group systems have been reported.**Objective:** We surveyed dogs for dog erythrocyte antigen (DEA) 1 and 2 new blood types (Kai 1 and Kai 2), and some samples also were screened for Dal and DEA 3, 4, and 7.**Methods:** Blood samples provided by owners, breeders, animal blood banks, and clinical laboratories were typed for DEA 1 by an immunochromatographic strip technique with a monoclonal antibody and analysis of band intensity. Both new antigens, the Dal and other DEAs (except DEA 7 by tube method), were assessed by a gel column method with either monoclonal or polyclonal antibodies. The same gel column method was applied for alloantibody detection.**Results:** Of 503 dogs typed, 59.6% were DEA 1+ with 4% weakly, 10% moderately, and 45.6% strongly DEA 1+. Regarding Kai 1 and Kai 2, 94% were Kai 1+/Kai 2-, 5% were Kai 1-/Kai 2- and 1% were Kai 1-/Kai 2+, but none were Kai 1+/Kai 2+. There was no relationship between Kai 1/Kai 2 and other blood types tested. Plasma from DEA 1-, Kai 1-, Kai 2- dogs, or some combination of these contained no detectable alloantibodies against DEA 1 and Kai 1 or Kai 2, respectively.**Conclusions and Clinical Importance:** The new blood types, called Kai 1 and Kai 2, are unrelated to DEA 1, 3, 4, and 7 and Dal. Kai 1+/Kai 2- dogs were most commonly found in North America. The clinical relevance of Kai 1 and Kai 2 in canine transfusion medicine still needs to be elucidated.**Key words:** Alloantibodies; Blood types; Dog erythrocyte antigen; Hemolytic transfusion reaction; Transfusion.

Each blood group system represents allelic surface red blood cell (RBC) antigens (types) that differ among individuals with >1% positive or negative dogs observed within a population. Alloantibody production can occur in individuals missing ≥ 1 of these antigens. Since the 1960s, >10 canine blood group systems have been reported.^{1–4} Because dogs do not appear to have any naturally occurring alloantibodies, these blood types were originally defined after experimental or accidental clinical sensitization of dogs by mismatched transfusions.^{3,5} In the mid-1970s, a workshop committee of the International Society for Animal Blood Group Research (now known as International Society of Animal Genetics) assigned 7 blood groups with the dog erythrocyte antigen prefix dog erythrocyte antigen (DEA).^{6,7} Subsequently, additional blood groups have been discovered, with Dal being the most recent and important,^{8–10} but they have not received an official designation. Moreover, it is difficult to determine whether all of them are indeed new, because the original cells and typing antisera are no longer available. None of the

Abbreviations:

DEA	dog erythrocyte antigen
EDTA	ethylene-diaminetetraacetic acid
Gel	gel column technique
LISS	low ionic strength solution
NaCl	sodium chloride
PBS	phosphate-buffered saline solution
RBC	red blood cells
Strip	immunochromatographic strip technique

canine blood group systems have been defined at the protein or molecular level thus far.^{1,3,4}

Clinically acute hemolytic transfusion reactions only have been reported in previously transfused dogs and only against DEA 1.1, 4 and Dal or unknown blood types.^{2,3,11,12} The DEA 1 blood group system, initially described with 3 types, DEA 1.1, 1.2 (and likely 1.3 [A3]), recently has been found, utilizing an anti-DEA 1 monoclonal antibody, to be a complex autosomal dominant allelic system, with a DEA 1- type and varied degrees of DEA 1 positivity from 1+ to 4+.^{13,14} In South Korea, additional blood group systems utilizing 2 new monoclonal antibodies, anti-Kai 1 and anti-Kai 2, currently are being investigated. The monoclonal anti-Kai 1 and anti-Kai 2 antibodies are of the IgM and IgG classes, respectively, and recognize different antigens of RBC membrane proteins in immunoblot studies. These monoclonal antibodies were utilized in the investigations reported here.

We surveyed the prevalence of 3 blood group antigens in a large group of dogs from North America with monoclonal antibodies against DEA 1, Kai 1, and Kai 2 and in a subset also compared those results with available antisera for other blood groups. Our results identified (1) the degree of DEA 1 positivity in a large canine population, (2) the presence of Kai 1 and Kai 2 in North American dogs, (3) the lack of a relationship of 2 new canine blood

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types, Kai 1 and Kai 2, to other blood group systems, and (4) the absence of alloantibodies in any type negative dogs before receiving transfusions.

Materials and Methods

Animals and Samples

Canine blood samples were obtained from owners, breeders, and blood banks or were made available as residual samples from the Clinical Pathology Laboratory at the Ryan Veterinary Hospital and were studied at the PennGen laboratory, University of Pennsylvania, from March to December 2015. Most samples originated from Philadelphia and the surrounding Tristate area (Pennsylvania, New Jersey, and Delaware) except for the Greyhounds and Dalmatians. The >1 mL ethylene-diaminetetraacetic acid (EDTA)-anticoagulated blood samples were kept chilled and typed within 10 days of collection. To standardize results, 20 and 1% RBC suspensions were prepared for each sample as previously described.¹³ The few DEA 7+ and DEA 7- blood samples were typed by ABRI.³ There were no specific selection criteria, but rather the samples that could be made available as previously typed by ABRI. The anti-DEA 7 antibody reagents were very weak and did not work satisfactorily in our hands. These studies were performed and approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

DEA 1 Typing by Immunochromatographic Strip

For DEA 1 typing, a commercially available immunochromatographic strip technique⁵ was used according to the manufacturer's instructions and as described previously by the PennGen laboratory, adjusting blood samples to a 20% RBC suspension.^{13,15} The results were captured by imaging,^c and the DEA 1 band strength was assessed on a scale of 0 (no band) to 4+ (as strong as control band) by visual and densitometric analyses by GeneTools software.^d

Kai 1, Kai 2, Dal, and DEA 3 and 4 Blood Typing by Gel Column (Gel)

Blood samples were centrifuged to pellet RBCs. Plasma was removed and stored frozen. The packed cells were washed 3 times with phosphate-buffered saline, each time discarding the supernatant. Then, 10 μ L of packed cells was added to a culture tube^e with 1 mL low ionic strength solution^f to prepare a 1% RBC suspension. We adapted the original tube assay for Kai 1 and Kai 2 to a gel column technique. Thereby, plain saline gel columns⁸ were used to detect agglutination reactions as previously described.¹⁶ Briefly, 25 μ L of antibody reagent (Kai 1, Kai 2, Dal provided by MC Blais, Montreal University) and DEA 3 and 4^h for extended typing (as well as saline as autocontrol), and 50 μ L of 1% RBC suspension were added on top of the gel and incubated at 37°C for 15 minutes in the manufacturer's incubator.^h Thereafter, the cards were centrifuged for 15 minutes at 85 \times g, with the manufacturer's centrifuge.ⁱ The degree of agglutination strength was graded from negative (0: all RBC at the bottom of the gel) to positive (4+: all RBC at the top of the gel). Results were interpreted as negative if \leq 1+ with practically all cells pelleted.

Alloantibody Detection in DEA 1-, Kai 1-, Kai 2- or Both Dogs' Plasma

For detection of alloantibodies in plasma samples, we used the gel as previously described.^{8,16} Briefly, we prepared 2–4 plain saline gel columns for each sample by placing 25 μ L of plasma from

Kai 1- or Kai 2- dogs and adding 50 μ L of Kai 1+ or Kai 1-, and Kai 2+ or Kai 2- RBCs in LISS, respectively. The results were graded as positive (majority of RBCs at the top of gel) or negative (majority of RBC at the bottom of the gel) as done with gel typing results above.

Results

This large typing survey included 503 dogs from North America representing 80 breeds and mixed breed dogs. A larger number of Dalmatians (N = 108 related to a separate Dal typing study), Greyhounds (70, mostly blood donors), and mixed breed (60) dogs were blood-typed, whereas all other breeds only had 1–25 dogs (median, 6) represented. Overall, there was no significant difference in blood type frequencies among breeds with at least 20 dogs typed, except for Dal- dogs (S. Goulet, U Giger, CC Euler, MC Blais et al., unpublished data, 2016). The typing survey results are summarized in Tables 1 and 2.

DEA 1 Typing and DEA 1 Alloantibodies

Utilizing the strip with a monoclonal anti-DEA 1 antibody and grading the DEA 1 band strength identified 40.4% DEA 1- dogs, with the remainder having varied degrees of DEA 1 positivity. Adjusting the PCV to 20% and quantitative analyses of the DEA 1 band strengths permitted differentiation of the degree of DEA 1 positivity (Fig 1). A large proportion of the DEA 1+ samples showed a very strong band (3+/4+), and only a small proportion exhibited 1+ and 2+ reactions. There was a close correlation between semiquantitative visual and densitometric assessment of the degree of DEA 1 positivity differentiating DEA 1- and weakly, moderately, and strongly DEA 1+ dogs. None of DEA 1- (and not previously transfused) dogs tested had any observable DEA 1 alloantibodies by gel.

Kai 1 and Kai 2 Typing and Alloantibodies

Monoclonal antibodies against red cell antigens were developed in South Korea, and 1 anti-Kai 1 and 1 anti-Kai

Table 1. Patterns of DEA 1, and Kai 1 and Kai 2 typing results among 503 dogs from North America.

Dogs		DEA 1	Kai 1	Kai 2
#	%			
282	56.0	+	+	–
191	38.0	–	+	–
15	3.0	+	–	–
10	2.0	–	–	–
3	0.6	+	–	+
2	0.4	–	–	+
Positive results				
#		300	473	5
%		59.6	94.0	1.0

DEA, dog erythrocyte antigen.

Table 2. Canine typing results of DEA 1, 3, 4, and 7 and Dal related to Kai 1 and Kai 2. Results <1+ are graded as negative.

	DEA 1		DEA 3		DEA 4		DEA 7 ^a		Dal	
	+	-	+	-	+	-	+	-	+	-
Kai 1+ and Kai 2-	282	191	1	79	133	0	15	8	5	5
Kai 1- and Kai 2-	15	10	5	7	24	1	0	0	5	5
Kai 1- and Kai 2+	3	2	0	2	4	0	0	0	0	5
Dogs #	503		94		162		23		25	

DEA, dog erythrocyte antigen.
^aExternal DEA 7 typing results.

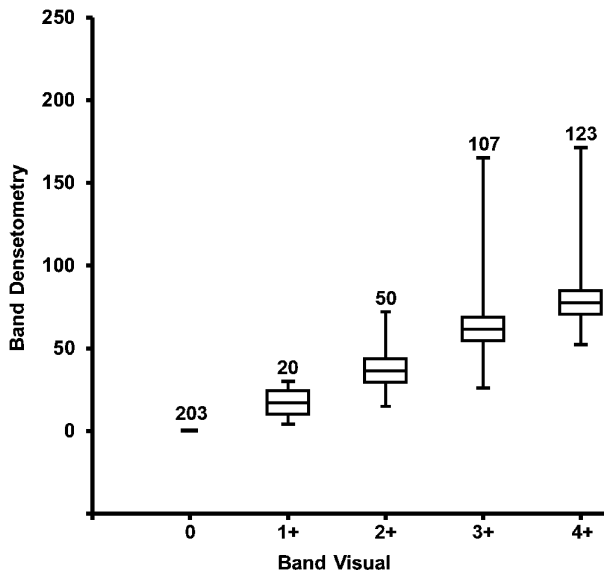


Fig 1. Visual and densitometric DEA 1 analyses of 503 dogs with immunochromatographic strip typing technique. Median, range, and extremes for each densitometric DEA 1 band strength compared to visual band assignment. DEA, dog erythrocyte antigen.

2 antibody (J.H. Lee, U. Giger, H.Y. Hee et al., unpublished data, 2016) were used in the survey reported here. We established a simple blood typing technique utilizing gel saline columns and adjusting the canine RBC quantity (1%) to compare the degree of the agglutination

reactions (Fig 2). The majority of canine blood samples typed Kai 1+ with most showing strong (3+/4+) and few moderate (2+) and weak (1+) agglutination reactions with the anti-Kai 1 reagent. In contrast, nearly all dogs typed as Kai 2- with only 5 dogs typed strongly Kai 2+, respectively (Table 3). In addition, 5% of the dogs were Kai 1- and Kai 2-, but most notably none were positive for both Kai antigens. Assessment of the plasma from all Kai 1- and Kai 2- dogs indicated no alloantibodies against Kai 1 and Kai 2 cells, respectively.

Extended Typing for DEA 3, 4 and 7 and Dal

To determine whether there was a relationship between the new Kai blood types and other blood groups, a subset of dogs were further typed by another laboratory for DEA 7 (in our laboratory, the available DEA 7 did not identify any agglutination reactions) or further typed for DEA 3, 4 and Dal with the Gel and available polyclonal antisera from negative dogs sensitized with positive red cells (Table 2). All dogs typed were DEA 4+ with 1 exception; this sole DEA 4- dog was a Dalmatian and typed as Kai 1-, Kai 2-, DEA 1+, DEA 3+, and Dal+. No relationship between DEA 1, 3, 4, and Dal and Kai 1 and Kai 2 could be detected. Furthermore, no relation between DEA 7 and Kai 1 could be found. Specifically, there were Kai 1- dogs that were DEA 1+, DEA 3+, DEA 4+ or Dal+. Similarly, the few dogs that were Kai 2+ could be DEA 1-, DEA 3-, or Dal-.

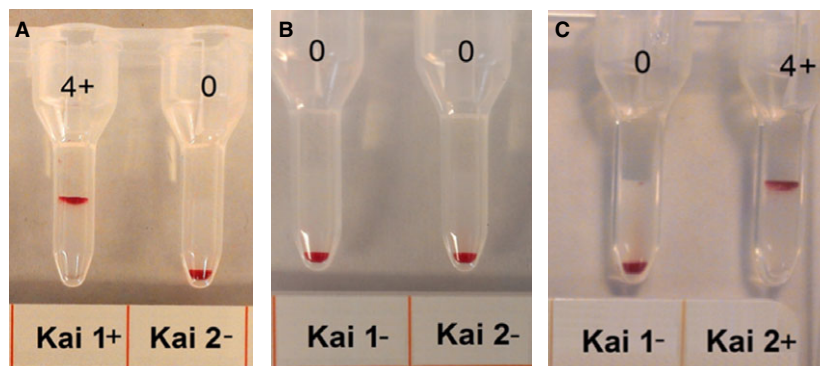


Fig 2. Gel column typing results showing the different Kai typing patterns for Kai 1 and Kai 2. (A) Kai 1+/Kai 2-, the most common pattern; (B) Kai 1-/Kai 2-; and (C) Kai 1-/Kai 2+. All autocontrols were negative for agglutination.

Table 3. Agglutination reactions for Kai 1 and Kai 2 typing with gel column. The degree of agglutination shows grading from negative (0: all of RBC at the bottom of the gel) to positive (4+: all of RBC at the top of the gel).

Agglutination	# Dogs	
	Kai 1	Kai 2
0	30	498
1+	0	0
2+	2	0
3+	76	0
4+	395	5

Discussion

Two monoclonal antibodies against red cell antigens recently were developed in South Korea. Based on their original preliminary evaluation, these 2 murine antibodies, named anti-Kai 1 and anti-Kai 2 (Kai refers to dog in Korea), recognize 2 different canine red cell antigens (J.H Lee, U. Giger, H.Y. Kim et al., unpublished data, 2016). To determine whether Kai 1 and Kai 2 exist in North America and are novel red cell antigens, we compared their expression to those of known blood types. We documented the presence of both Kai 1 and Kai 2 in North America. As far as we could determine, neither Kai 1 nor Kai 2 is associated with each other (albeit we did not find any Kai 1+/Kai 2+ dogs) nor with any known canine blood group system. Both Kai 1- and Kai 2- dogs as well as DEA 1- dogs lacked any naturally occurring alloantibodies against Kai 1 and Kai 2 and DEA 1, respectively. The clinical importance of these new blood groups in transfusion treatment still remains to be determined.

In our survey of 503 dogs mostly from Philadelphia and the Tristate area, including a relatively large number of Greyhounds (70 samples) and Dalmatians (108 samples) from the USA and Canada, 94% of dogs were Kai 1+ but only a few (1%) were Kai 2+. Therefore, the combination of Kai 1+ and Kai 2- was most frequently (94%) observed. The Kai 1+/Kai 2- blood type constellation was found in every blood donor Greyhound tested, whereas in other breeds tested, Kai 1+ and Kai 1- dogs were found. Among the few Kai 2+ samples, 3 were from Lhasa Apsos. Since completion of these studies, a limited preliminary survey from the United Kingdom also found several Lhasa Apsos with Kai 1-/Kai 2+ blood (Watson, C. C. Euler and U. Giger, unpublished data, 2016). Similarly, a recent preliminary survey of mastiffs in South Korea indicated a 17% Kai 2+ frequency (J.H. Lee and H.Y. Kim et al., unpublished data, 2016). Another possible constellation found was Kai 1-/Kai 2-, whereas no dog was Kai 1+/Kai 2+. Results of limited family studies are consistent with an autosomal dominant trait for Kai 1 (J.H. Lee and H.Y. Kim et al., unpublished data, 2016). Because the frequency of the 2 phenotypes Kai 1+/- and Kai 2+/- are <99% and ≥1%, respectively, Kai 1 and Kai 2 should be considered blood groups rather than high-

low-frequency (public or private) red cell antigens.^{17,18} DEA 4 was thought to be a blood group, although ≥99.5% of dogs seem to be DEA 4+ and thus, it should be considered a high-frequency antigen. In contrast, Dal was thought to be a high-frequency red cell antigen with Dal- originally only found in a few Dalmatians, but larger surveys found Dal- dogs in Dobermans and other breeds and thus, it should be recognized as a blood group.⁸⁻¹²

Blood typing for Kai 1 and Kai 2 was determined by traditional tube agglutination assays in South Korea. We adapted the gel column technique for Kai 1 and 2 typing and alloantibody studies with neutral NaCl gel column cards, which can be better standardized and are simple to perform and interpret, as we have previously shown for DEA 1^{13,19} and other DEA types¹⁶ as well as Dal types.^{9,10} The degree of agglutination was very strong (3+ or 4+) for both Kai 1 and Kai 2 antigens in nearly all cases with a few samples giving weaker reactions (2+). Moreover, the results were stable during repeat typing, as previously shown for DEA 1,¹³ and results were easy to interpret. However, we did have a few cases in which we observed an unexplained split reaction, with the majority of cells located on top of the gel despite having a few RBCs pelleted; we designated such cases as 4+. These dogs were neither previously transfused nor had any illness. Overall, these monoclonal antibodies and current gel column typing (and tube) techniques seem well suited to detect positive agglutination reactions for screening dogs for the Kai 1 and Kai 2 blood types in clinical settings. Commercial Kai typing kits may be developed in the future, if there is more clinical evidence for blood incompatibilities and transfusion reactions.

The DEA 1 blood group system has not been defined at the protein and molecular levels, but based upon recent studies seems to originate from a single locus with ≥4 alleles, and thus DEA 1- and weakly to strongly DEA 1+ dogs rather than DEA 1.1, DEA 1.2 and DEA 1.3 types.^{1,2,13} We have shown previously that DEA 1 typing with a monoclonal antibody by gel column, flow cytometry, and chromatographic strip produced identical results.¹³ In this survey of 503 dogs, we applied the chromatographic strip method with visual and densitometric grading at PCV 20% as previously described.¹³ The proportions of DEA 1- and strongly DEA 1+ (3+/4+) dogs were far larger than those that were weakly (1+) or moderately (2+) DEA 1+. Among breeds with at least 25 dogs represented, DEA 1- and DEA 1+ dogs were found, which is consistent with our prior smaller surveys^{13,14} and also reports with DEA 1.1 and 1.X polyclonal antisera.²⁰ However, our survey included a biased group of dogs, because it included many Greyhound blood donors from 2 animal blood banks, where DEA 1- dogs are preferred, and many Dalmatians related to a separate Dal typing survey (S. Goulet, U. Giger, J. Arsenaault, A. Abrams-Ogg, C. C. Euler, and M.-C. Blais. et al., unpublished data).^{9,10} Finally, consistent with prior observations,³ plasma from the previously untransfused DEA 1- dogs screened indicated a lack of naturally occurring anti-DEA 1 alloantibodies.

Results of the Dal typing survey of the dogs in our study will be presented elsewhere, but as expected based upon small prior surveys, Dal- dogs were found among Dalmatians and Doberman Pinschers, Shih Tzus as well as in few other breeds (S. Goulet, U. Giger, J. Arsenaault, A. Abrams-Ogg, C. C. Euler, and M.-C. Blais. et al. unpublished data, 2016).^{9,10} However, these frequencies of Dal- dogs may not be representative because the number of dogs tested per breed was small and owners and breeders of Dal- dogs likely submitted samples from related dogs in order to find potential blood donors for their Dal- dogs. Again, the Dal blood group system has no relationship to the new Kai 1 and 2 blood group systems.

Comparison of the DEA 1 typing with Kai 1 and Kai 2 typing results in our survey indicates that these are different blood group systems. Reagents are no longer available for all previously reported canine blood group systems, such as DEA 5, 6, and 8. These DEA canine blood groups and alloantibodies had not well been characterized (mostly based upon agglutination reactions),^{15,18} and consequentially, it will be impossible to determine whether the antigens Kai 1, Kai 2, or both are related to any previously described blood groups. However, based upon our limited extended typing comparisons, there is no relationship to the known canine blood groups for which reagents were available (DEA 1, 3, 4, 7, and Dal). Thus, Kai 1 and Kai 2 appear to be newly discovered red cell antigens. Moreover, a direct relationship between Kai 1 and Kai 2 could not be determined, although there were no dogs positive for Kai 1 and Kai 2. However, a few dogs were Kai 2+ and based upon immunoblotting studies utilizing the 2 monoclonal antibodies, the sizes of the 2 red cell antigens are different (J.H. Lee, U. Giger, H.Y. Kim et al., unpublished data, 2016).

These DEA and Kai 1 and Kai 2 typing surveys and alloantibody studies have clinical implications. From a practical perspective, it is highly advantageous that most dogs are either clearly DEA 1- or strongly DEA 1+ and similarly clearly negative or positive for either Kai 1 or 2 type. The occasional weakly to moderately DEA 1+ dog is still important to identify. Currently, it is not known whether weakly to moderately DEA 1+ cells will induce alloantibodies in DEA 1- recipient dogs and whether these weakly to moderately DEA 1+ dogs would be sensitized by strongly DEA 1+ blood. Thus, specific evidence-based recommendations regarding those cannot be made yet. We continue to follow the cautious practice of typing donor and recipient, giving DEA 1- and also weakly DEA 1+ recipient dogs only DEA 1- blood, and labeling weakly or moderately DEA 1+ donors as DEA 1+ donors.^{3,13} Recommendations for Kai must await demonstration of any alloantibody development after transfusions. Preliminary unpublished experimental studies show the development of anti-Kai 1 and anti-Kai 2 alloantibodies in Kai 1- and Kai 2-dogs, respectively (J.H. Lee and H.Y. Kim et al., unpublished data, 2016).

The lack of naturally occurring alloantibodies against DEA 1 and both Kai antigens further supports the

practice of not crossmatching before a first transfusion.³ Although acute hemolytic transfusion reactions have been documented in previously transfused dogs due to DEA 1, DEA 4, and Dal incompatibilities,^{2,8,11,12} there is no published evidence of alloantibody production and acute hemolytic transfusion reactions due to Kai-mismatched transfusions. Indeed, we used monoclonal murine antibodies against Kai 1 and Kai 2 and not anti-Kai alloantibodies from previously transfused dogs for typing. However, unpublished clinical results after Kai-mismatched transfusions indicate the development of anti-Kai 1 and anti-Kai 2 alloantibodies and hemolytic reactions (J.H. Lee and H.Y. Kim et al., unpublished data, 2016). Therefore, it appears appropriate to follow up dogs with incompatible crossmatches for Kai-type mismatches and potentially select donors based also on the Kai types in previously transfused dogs.

In conclusion, we established a simple and accurate gel column typing technique for Kai 1 and Kai 2 and found both red cell antigens with most dogs to be Kai 1+/Kai 2- in North America. These blood types represent novel and distinct blood groups with potentially different frequencies among breeds. Dogs negative for Kai 1, Kai 2, or both do not have any naturally occurring alloantibodies in their plasma. The clinical importance of Kai 1 and Kai 2 in canine transfusion medicine needs to be determined.

Footnotes

^a ABRI, Stockbridge, MI

^b "Strip", Alvedia, Lab Test DEA 1, Limonest, France

^c Syngene G:Box, Syngene USA, Frederick, MD

^d Syngene USA

^e Fisher Scientific, Pittsburgh, PA

^f LISS, ID-Diluent 2, DiaMed GmbH, Cressier, Switzerland

^g "Gel", BioRad ID-Cards, NaCl, Enzyme Test and Cold Agglutinins DiaMed

^h ID-Incubator 37 S I, DiaMed-ID, Microtyping System, DiaMed GmbH

ⁱ ID-Centrifuge 12 S II, DiaMed-ID, Microtyping System, DiaMed GmbH

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Conflict of Interest Declaration: The authors at the University of Pennsylvania (PennGen) offer blood typing services. The authors in South Korea are associated with the Korean Animal Blood Bank.

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